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Letter to the Editor

Biological Effects of Fibers: Stanton's Hypothesis Revisited

by Jacques Dunnigan*

In the current American best-seller, *IN SEARCH OF EXCELLENCE, Lessons from America's Best-Run Companies*, co-authors Thomas J. Peters and Robert H. Waterman are strongly advocating a drastic change in corporate mentality, as well as a courageous re-vamping of American business schools curriculum:

> "We really are talking about what Thomas Kuhn, in his landmark book *The Structure of Scientific Revolutions*, calls a *paradigm shift*. Kuhn argues that scientists in any field and in any time possess a set of shared beliefs about the world, and for that time the set constitutes the dominant paradigm. What he terms "normal science" proceeds nicely under this set of shared beliefs. Experiments are carried out strictly within the boundaries of those beliefs and small steps toward progress are made. An old but excellent example is the Ptolemaic view of the universe (which held until the sixteenth century) that the earth was at the center of the universe, and the moon, sun, planets, and stars were embedded in concentric spheres around it. Elaborate mathematical formulas and models were developed that would accurately predict astronomical events based on the Ptolemaic paradigm. Not until Copernicus and Kepler found that the formula worked more easily when the sun replaced the earth as the center of it all did an instance of paradigm shift begin. After a paradigm shift begins, progress is fast though fraught with tension. People get angry. New discoveries pour in to support the new belief system (e.g., those of Kepler and Galileo), and scientific revolution occurs. Other familiar examples of paradigm shift and ensuing revolution in science include the shift to relativity in physics, and to plate tectonics in geology. The important point in each instance is that the old 'rationality' is eventually replaced with a new, different, and more useful one. We are urging something of this kind in business."

In the very specialized field of the mechanism of action of fibrous particles at the cellular level, such a "paradigm shift" seems to be taking place, and again the shift is not being operated without its expected tensions. Like it or not, it does seem that scientists are having a second look at what has been called "Stanton's Hypothesis." Simply stated, Stanton's explanation proposed that the biological effects of fibers "depend on dimension and durability rather than physicochemical properties." This hypothesis was formulated, in the early '70s, as a result of observations associating dimensional distributions of eight different durable fibrous minerals by varying degrees of efficacy in inducing malignant mesenchymal neoplasm when implanted in the pleurae of female Osborne-Mendel rats. It was found that neoplastic response correlated well with the dimensional distribution of fibers. "The strongest correlation was found with fibers that measured $\geq 0.25\mu$ in diameter and 8μ in length, but fibers in adjacent size ranges up to 1.5μ in diameter and greater than 4μ in length also correlated to a lesser degree with tumor incidence." These observations were reported in a review paper which appeared in Advances in Oncology in 1978 (*1*). One very important point which must be noted from this paper deals with the *methods* applied by these authors to obtain the fibers used in their experiments: "Various methods of processing the fiber samples, including ball-milling, centrifugation, and filtration, were used in an attempt to achieve a diversity of dimensional distribution of fibers." I will return to this aspect later on, but let us take note right now of the very important aspects raised by the actual comminution methods used in order to obtain fiber samples of different sizes.

Let us now consider other data which, in the course of the last decade, pointed to the possibility that at least part of the biological effects could be associated with some chemical surface characteristics, as opposed, or complementary to fiber dimension. In the proceedings of a meeting (*2*) on the *in vitro* Effects of Mineral Dusts, which took place in Penarth (U.K.) in 1979, Bignon and his colleagues from Paris reported their studies on the significance of lysosomal enzyme release induced by asbestos, and showed how their results are related to the shape, chemistry and the surface properties of the fibers. They indicated that their results showed the importance of the Si-OH group on the determination of the minerals' effects on alveolar macrophage. They concluded: "From the results reported here, it appears that the biological effects are related to the surface properties rather than to the shape of the fibers." The same group mentioned their previous papers, which suggested that haemolysis may be due to an adsorption of the membrane constituents onto the chrysotile fibers. After recalling the suggestion by Harington (*3*) in 1971 that "one explanation of chrysotile haemolysis could be that membrane sialoglycoproteins interact with chrysotile fibers," and the mention (*4*) by Allison in 1972 that "proteins form clusters beneath the fibers which create channels, through which small molecules are able to move freely..." and that "...haemolysis would thereafter occur due

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to osmotic swelling and bursting of RBC," Bignon proposed that " . . . phospholipids and membranes are adsorbed, and this may induce lysis."

At the same meeting, Professors Sykes, Morgan and Holmes of Harwell, Oxfordshire (U.K.) mentioned (*5*) that their studies on the "characteristics of chrysotile haemolysis support the theory of protein clustering and osmotic lysis" proposed by Harington (*6*) in 1975, and further added that "The opposing characteristics of quartz haemolysis suggest the existence of a different mechanism which may involve its binding to either lipids as suggested" by Nash (*7*), or "quaternary ammonium groups in the membrane," as proposed by Depasse (*8*). It is clear that whatever the molecules involved, all these authors specifically pointed to a chemical rather than a geometric or physical factor as the important determinant in the cell membrane effects.

Another important contribution (*9*) at that meeting was also made by Miller of the National Center of Occupational Health of South Africa. She presented evidence that was in accordance with the theory that "Recognition by phagocytes may be due to physicochemical affinities between the cell and the recognized material," as proposed in 1976 by Wilkinson (*10*), as well as with the mention (*11*) by Stossel in 1972 that "the ingestibility of particles may be influenced by variation in their net surface charges or their hydrophobic properties."

From their studies published in 1977 (*12,13*) Light and Wei, from the U.S., reported that "surfactant readily adsorbed onto the surface of fibers, causing a decrease in the zeta potential, and a corresponding decrease in hemolytic activity." At the 1979 meeting in Penarth, they proposed that "Properties which have been considered to be primary determinants of biological effects of asbestos are: surface charge, solubility, specific surface area, and fiber dimensions." On the basis of their results, they proposed that the most important of these properties is surface charge. They added that "fiber dimensions are important in determining whether asbestos fibers are able to reach sites where critical cellular interactions take place, and thus, could govern whether the potential biological activity of fibers due to their surface charge is displayed" (*14*). Finally Newman, Saat and Hart from the College of Medicine of Ohio State University reported the results of their studies on hamster embryo cell membrane alterations by asbestos fibers (*15*). They concluded that: "Thus asbestos may have induced some changes in the plasma membrane similar to those evidenced in transformed cells and since asbestos has not been demonstrated to be a mutagen," (*16*) "the rôle of asbestos in the mutagenic process may be to modify the cell surface so as to give carcinogens and viruses greater access to the cell nucleus and, thus, to promote cell transformations." This very rapid and certainly incomplete review of recently published data by different authors of various laboratories throughout the world point to the crucial need to re-assess what had been called Stanton's Hypothesis. It is possible that the work of Stanton and the resulting hypothesis, might have been the result of the presumption that all comminution methods are equally good in the preparation of fiber samples of different sizes and that all they do is merely reduce the dimension of fibers.

That such is not the case—especially when ball-milling is used—has been demonstrated by at least two authors. For instance, Langer in 1978 showed that ball-milling of experimental samples results in important changes in the structural and surface characteristics of asbestos fibers, and reduces their effects on cell membrane (*17*). This was a most important observation, in view of the fact that for many years, the dominance of "long" fibers in pathogenicity has been regarded as established, and that most original studies claiming that short fibers lack fibrogenic activity, used traumatic ball-milling preparatory steps. Studies using less vigorous preparatory techniques, such as flotation for instance, have yielded positive results. This is probably what led Langer to conclude that "The hypothesis that particle shape is the major etiologic mechanism in fibrotic or carcinogenic responses appears to be somewhat oversimplified."

Finally, Spurny in Germany recently published essentially the same observation, and concluded that "milling procedures change not only the size distribution but also the shape and crystal structure of asbestos fibers. They are therefore not recommended as comminution methods for preparation of fibrous material used in biological experiments" (*18*). Unfortunately, this is precisely what Stanton and others did, at least with part of their prepared samples. While it should not be construed from this that Stanton's Hypothesis should be totally rejected, the time has come to at least take a second look, and indeed may well call for a paradigm shift.

There are now additional reasons to do this, and they stem from the development of the concepts of initiation and promotion, related to the multistep process of carcinogenicity. This has resulted in efforts to classify agents as genotoxic and epigenetic, depending on their mode of action. It is generally recognized that carcinogenesis is a multistep process which occurs mainly in two sequential stages: initiation and promotion. The initiation stage corresponds to some induced alteration in the cell, associated with a damaged or modified DNA replication system. The promotion stage encompasses a number of conditions necessary for malignancy to be expressed in an "initiated" tissue. This scheme has formed the basis for the distinction of chemical agents into two categories: those which damage genetic material directly: the so-called genotoxic agents and those that operate by indirect, non-genotoxic, or epigenetic mechanisms. A brilliant review on the subject has been published recently by Weisburger and Williams (*19*). The authors explain that genotoxic agents undergo a series of competing reactions, ultimately reacting with DNA, which appears to be the critical event in carcinogenesis. Once cell duplication with the so-generated abnormal DNA has occurred, the effect is basically irreversible. In contrast, the action of agents

operating by epigenetic mechanisms, which are as yet unclear and require much more research, usually necessitates their presence at high levels for a long time and, indeed, is reversible up to a certain point.

Substances operating on cell systems as epigenetic agents act by diverse mechanisms that are definitely different from those involving genotoxic pathways. A case in point is the situation where numerous well-known genotoxic agents have been used experimentally, both *in vivo* and *in vitro*, in combination with asbestos fibers. One of the best studied agents is B(a)P (benzo-α-pyrene). It has been reported by many authors that the mutagenic and carcinogenic potentials of this chemical are enhanced considerably when associated with particles. According to some authors, this promoting effect of particles could be related to the fact that when B(a)P is adsorbed onto the particles, there is a resulting enhanced transport and uptake of the carcinogen into microsomal membranes (*20,21*). The resulting effect of such particle-enhanced transport of carcinogens has been illlustrated again recently by Reiss et al. (*22*), who studied the comutagenicity of chrysotile asbestos and B(a)P. The authors found that exposure of adult rat liver epithelial cells to chrysotile alone did not increase the mutant incidence, whereas B(a)P was mutagenic. Simultaneous exposure of the cells to chrysotile and B(a)P resulted in a greatly enhanced mutant recovery compared to either of these substances alone. The authors indicate that these results extend their previous studies and strengthen the proposal that asbestos is not a genotoxic carcinogen capable of altering DNA. Using a different cell bioassay, Poole et al. (*23*) have come to essentially the same conclusions. In their study these authors investigated the cell-transforming ability of amphibole asbestos dust, using C3H10T fibroblasts. Again, it was found that crocidolite and amosite cause no increase in the number of transformed colonies over that seen in cultures from untreated cells, but the dusts were able to enhance substantially the oncogenic effect of B(a)P.

These results are essentially consistent with the previously expressed views of Mossman and Craighead (*24*). These authors found that aryl hydrocarbon hydroxylase (AHH) activity was not changed in hamster tracheal epithelial organ culture after exposure to crocidolite (AHH system is required to metabolize a procarcinogen into a reactive, or "ultimate" carcinogen). However, it was observed that crocidolite fibers potentiated the effects of 3-methylcholanthrene (3-MC). Consistent with these results, the authors found that carcinomas developed after 12 to 52 weeks from tissues exposed to crocidolite with surface-bound 3-MC, whereas neoplasms failed to evolve from organ cultures exposed to crocidolite in the absence of 3-MC (*25*). The authors suggest that "asbestos fibers might serve as a physical carrier of chemical carcinogens, providing a means of introducing polycyclic hydrocarbons into the cells, assuming these chemicals are adsorbed to the fibers before phagocytosis." Furthermore, they suggest that crocidolite, due to its chemical constitution, might also have a direct, potentiating effect on the AHH system. Thus in many respects, asbestos resembles a classical tumor promoter. In partial contradiction to the effect just reported by Mossman and Craighead on AHH inducibility, it may come as a surprise to learn that crocidolite and chrysotile have been found to inhibit AHH (*20*); these authors intrepret their finding as yet another mechanism, whereby asbestos fibers would retard the rapid metabolism of B(a)P, and thus prolong the retention of the carcinogen in the tissue, thereby increasing the risk of induction of carcinoma. Clearly, these opposite views in the data, and the ensuing interpretation in terms of mechanism of action, will have to be sorted out.

However, both groups of workers are in total agreement as to the importance of the phenomenon of adsorption of B(a)P on asbestos fibers. Moreover, most reports dealing with this phenomenon are in agreement to underline the compelling relevance of this observation to epidemiological data, which clearly indicates an association between exposure to asbestos dust and the high incidence of lung carcinoma in smokers. For instance Selikoff et al. (*26*) have established that the incidence of pulmonary cancers among asbestos workers who are cigarette smokers is 92 times that of the general population, whereas the increase in disease among nonsmoking asbestos workers is quite low. This particular situation where the experimental data correlate so remarkably well with epidemiological data is literally begging the question, which can be put in rather simple terms: which parameter is important in the adsorption phenomenon of B(a)P on asbestos fibers? Is it size? Or is it chemical constitution?

The obvious answer to this question is that while surface area of particulate materials will influence the quantity of molecules adsorbed on reactive sites, the phenomenon in and by itself will not be greatly influenced by the geometry of the particles. Chemical constitution of the adsorbent however will be the determining factor whether a substance will be adsorbed or not.

And so, it stands to reason that while particle size will determine whether airborne fibrous particles will eventually reach the pulmonary airways, both experimental evidence and epidemiological data would support the view that there is a tremendous difference in risk of lung cancer between a particle carrying a genotoxic agent such as B(a)P, and a particle which is not chemically apt to act as "carrier." According to both experimental (*21–25*) and epidemiological data (*26*), this difference would be severalfold in magnitude. This sole fact brings ample justification to re-affirm that for respirable particles, chemical constitution—more specifically the potential of a particle to act as a carrier (and possibly as an AHH inducer)—is of paramount importance in determining the potential degree of risk for lung carcinoma.

This view in no way minimizes other mechanisms which may be operating when particles come in contact with tissues. Cell membranes may be affected adversely

by the so-called cytotoxic properties of particles. For some authors, this in itself could eventually trigger the process known as solid-state carcinogenesis. Mesothelioma, which is not known to be associated with tobacco (*27*), could be more specifically due to this second type of mechanism. But even in this situation, experimental evidence has shown that the cytotoxic potential of particles can be altered by chemical modifications (*2*), with resulting modifications in the potential of such modifications for inducing mesothelioma (*28*). This led these authors to state that ". . . size is not the only factor involved in the induction of pleural cancers by mineral fibres."

Finally, an important study was published by Poole et al. (*29*), showing that, contrary to asbestos fibers which have been shown to be epigenetic but not genotoxic, fibrous erionite displays genotoxic properties. Their data further weaken the fiber size theory, by showing that fewer erionite fibers (150 f/μg) of so-called pathogenic size range are far more active than a larger number (1.6×10^5 f/μg) of similarly sized crocidolite fibers. These authors recall the results of Suzuki (*30*), Wagner (*31*) and Maltoni et al. (*32*), showing fibrous erionite as possibly the most powerful mesothelioma-producing agent ever found. They suggest that the fiber size theory may be incorrect, that erionite fibers are qualitatively different, and that they might display adsorptive or catalytic properties which are not shared equally among mineral fibers.

Conclusion

Surely, the complexity of the mechanisms involved warrants further investigation to better understand the complementary relevance of size and chemical constitution in the interaction of particles and cells. But consideration of the fact that the co-carcinogenic effect of asbestos with cigarette smoking is by far the main health hazard, chemical constitution of particles, which underlies this phenomenon, would appear to be of singular importance. It follows from this that cytotoxic respirable particles which adsorb and carry genotoxic agents, such as B(a)P, carry a far greater health hazard than less cytotoxic particles, which do not adsorb and carry such agents. This could possibly serve as an element of a strategy for the design and development of safer inorganic fibers.

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